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INTRODUCTION

This technical bulletin summarizes presentations made at the October 17, 1989 Salmon Genetics Workshop, which was initiated to provide a forum for discussions on Atlantic salmon and similar species. The suggestions and innovations presented in these papers and the discussions which ensued all pertain to current and future projects in the Salmon Genetics Research Program (SGRP). The exchange of ideas greatly enriched the SGRP review process undertaken by the Scientific Advisory and Steering Committees on the two days following the workshop. The SGRP staff are grateful to the speakers for their contributions and to the Department of Fisheries and Oceans for providing the facilities.

RESEARCH OPTIONS RELATED TO WILD SALMON POPULATIONS

Dr. W.M. Carter, Atlantic Salmon Federation (ASF)

HISTORICAL BACKGROUND

The original proposal to initiate the Salmon Genetics Research Program (SGRP) concept came from an International Atlantic Salmon Federation (IASF) Scientific Advisory Group meeting in Hobe Sound, Florida, January 7 and 8, 1971. Funding was approved for a Planning Study for "a cooperative research program to rear selected strains of Atlantic salmon in an experimental hatchery. The purpose would be to develop strains of salmon genetically adaptable to a specific freshwater environment. Subsequently, in cooperation with government agencies in the northeastern states and Canada, these special strains would be used to assist the rehabilitation of salmon rivers." Later, in 1973, the fund-raising brochure prepared by Dr. John Calaprice described the project as "a center for breeding, education, and river rehabilitation programs."

The SGRP was the third major program area entered into by IASF, and perhaps its most ambitious. The first and second SGRP objectives, breeding and education, came quickly and have expanded steadily since inception of Phase I of the program in the spring of 1975.

Objective 1. Breeding

One only needs to read the SGRP Annual Reports or the numerous published papers by SGRP scientific staff to appreciate the enormous wealth of breeding data the SGRP has added to previously existing knowledge. A principal beneficiary has been the salmon aquaculture industry in the Bay of Fundy region of New Brunswick. The industry might still have appeared without SGRP influence, but could it have developed as rapidly and successfully? Of more direct interest is the fact that contributing to the establishment of an aquaculture industry was not even part of the original concept.

Objective 2. Education

a) A footnote of historical interest is that the SGRP, because of the need to staff its research hatchery, was responsible for the concept and initiation of the New Brunswick Community College's Aquaculture Technician

Training Program (ATTP). This program has played a large part in the success of the aquaculture industry by providing technically trained fish culturists. Some may not realize that many of the most successful salmon farmers in New Brunswick and in other locations, including the United Kingdom, gained their education and/or training through SGRP and ATTP.

b) Cooperative research:

SGRP facilities and programs opened doors for many individuals in different institutions to expand their level of knowledge. This may be one of the most significant educational contributions of the SGRP. Perhaps we should look more closely to the cooperative research area for options to initiate new research that would benefit wild stocks.

c) Seminars and Publications:

The list is long and impressive. Through these communications, the information generated by the SGRP has been disseminated to every corner of the Atlantic salmon world.

d) Visiting Scientists:

Visitors to the SGRP have been numerous and their home areas varied. Early in October 1989, a delegation from the USSR visited the SGRP and were introduced to information which will be relevant and helpful to their domestic research activities. We did not think, in June 1975, that the SGRP would one day play host to leading fishery scientists from the USSR.

Objective 3. River Rehabilitation:

Progress in this area is more difficult to document. Perhaps the original concept - to develop strains of salmon genetically adaptable to specific freshwater environments - may have been overly ambitious and perhaps even genetically naive. Direct application of the SGRP knowledge bank to natural stocks has proven very difficult.

It may be that the indirect benefits of SGRP research and educational activities in the areas of aquaculture

production, fish husbandry techniques and selection for hatchery rearing will eventually be the most significant SGRP contribution to the management of wild stocks. However, these benefits are difficult to quantify. Consequently, the program sponsors have been called upon too frequently to justify the commitment of substantial funding from the sport-fishing constituency and they have not had an easy time doing it. Without the aquaculture component to share the SGRP financial burden, and general support for this new industry, it may have proven impossible to maintain the financial support by one of the principal SGRP partners. There is double irony in this, because aquaculture was not even part of the original goals.

RESEARCH OPTIONS OF IMPORTANCE TO WILD STOCK MANAGEMENT

There are a number of areas where research can assist wild stock management objectives. Not all of them fall within the SGRP mandate, but it may be possible to address these exceptions through cooperative research.

1. Stock Identification:

   An accurate method of identifying river or area of origin would be helpful to managers. During North Atlantic Salmon Conservation Organization (NASCO) discussions seeking to reduce the Greenland quota in 1988, Canada argued that the North American component of the Greenland catch in 1987 had come primarily from Canada's southeastern rivers, where native stocks were already in deep trouble and where all users were severely restricted. Our arguments were weak because we did not have good stock identification evidence. Why is it easier to identify discrete river stocks of Pacific salmon? Can SGRP research help to develop a stock identification method for individual Atlantic salmon stocks?

2. Gene Bank:

   Atlantic salmon populations have been variously affected by civilization. As a consequence, some unique genetic strains have been lost. Laval River in Quebec is a good example. Laval River was home to a small population of salmon with distinct characteristics. Returnees were exclusively 3-sea-winter fish that weighed

no less than 18 and no more than 22 pounds. Poaching and other factors gradually diminished the numbers, until eventually the population was extinct and this unique genetic material lost forever.

Growth of aquaculture and introduction of new genetic strains through straying may lead to further losses of wild stocks. Once the original genetic material is extinct, it is irreplaceable. Should the SGRP be developing a Canadian Atlantic salmon gene bank program for wild stock, as is being done for aquaculture broodstock? Is there something to learn and time to be saved by studying the gene bank program in Norway?

3. Genetic Impact on Migration:

One of the original dreams of SGRP planners was to develop a strain of grilse (or salmon) which did not migrate to high seas interception areas, where "fair share" laws require that stocks must be shared fairly with countries in the destination area. Is this idea completely dead and is the dream genetically impossible? If a population could be developed which exhibited a short migration pattern, how could this be introduced into wild runs? Inner Fundy stocks, for example, exhibit a shorter migration pattern, overwintering principally in the Bay of Fundy and the Gulf of Maine.

4. Disease and Parasite Resistant Strains:

Furunculosis is a serious problem in some wild stocks. A notable example in Canada is the Restigouche. In Norway, the introduction of Gyrodactylus salaris has resulted in severe damage to wild populations.

Resistant strains occur naturally. Baltic salmon harbour Gyrodactylus salaris without harm, but when the pathogen was introduced to Norwegian salmon, the effects were severe. Is it practical and feasible for SGRP to devote more attention to research on disease and parasite resistant strains and development of a protocol for their use in wild populations?

5. River/Zone Management:

Canada is moving toward a river management policy for salmon management, and will test several rivers for the introduction of river management techniques by 1990. In the ideal situation, quotas will be set for all fisheries on most individual river systems. Differentiations between early and late run stocks may be an important aspect of quota management. Is there a genetic difference between early and late run salmon? If so, how can early and late run fish be identified? Is there support for the hypothesis that early run salmon run further upstream than late run fish and consequently there is little or no crossing between them? Can this explain the gradual loss of resident populations in lower section pools on many rivers such as the Matapedia?

6. Grilse vs. Salmon:

Do we have a single, universal definition of and explanation for grilsification in wild populations? How could grilsification be influenced in wild populations? What is the normal composition of a salmon family which results from grilse x grilse, grilse x salmon and salmon x salmon matings?

7. Genetic Dilution:

Are we completely satisfied that cross breeding between aquaculture escapees and wild stocks will not result in severe genetic damage - even extinction of wild populations in some river systems? In Norway a very high proportion of the total run in some rivers is comprised of escapees. It is therefore reasonable to conclude that crossing is taking place. Do we have enough information to control genetic dilution in wild populations? Should the SGRP try to prepare a list of specific, practical recommendations, such as quality standards for cages or use of sterile or native salmon for cage culture, which can be implemented and enforced to reduce the impact of salmon aquaculture on wild stocks? The bilateral working group list may be incomplete or impractical.

OPPORTUNITIES FOR THE SGRP IN RESEARCH RELATED TO ATLANTIC SALMON AQUACULTURE

Blair Moffatt[1], Sea Farm Canada, Inc., P.O. Box 2030, Saint John, New Brunswick, E2L 3T5

THE PRESENT

The SGRP has a selling job to do. Industry must be convinced of the ever-increasing value of the SGRP and its research to their goals of improved performance and bigger profits. Increases in productivity mean increased profits, and as such, represent a common ground between the industry and the SGRP which has been responsible for improvements in aquacultural stocks through breeding.

The quality of New Brunswick farmed salmon must be promoted to the public. The general trend is toward greater seafood consumption, in spite of a five percent drop last year in the United States, which was blamed on the public's concern about pollution of the marine environment and its effects on the safety of seafood. Consumers must be educated to the health benefits of New Brunswick's salmon. For example, Atlantic salmon have very low cholesterol levels and the highest Omega-3 fatty acid levels of all aquaculture species. The SGRP can play a role here.

Aquaculture is a young industry world-wide, but especially here in New Brunswick. We remain small players on the world stage. In terms of total production, we can't compete with the large producers like Norway, Scotland and Chile, but in terms of quality of environment and quality of product, we are on top. The Bay of Fundy region of New Brunswick and the Cobscot Bay in Maine offers some of the best rearing areas in the world. The severe winters appear to reduce the grilse rate and improve the quality of the end product.

The large producer countries, such as Norway and Scotland, have been experiencing serious problems. Scotland's grilse rate is one of the highest, ranging between 20 and 50 percent. Since costs involved in rearing the salmon are not covered until the fish reaches approximately 5 pounds, selling a 5-6 pound fish does not provide much of a profit margin. Norway has had serious smolt

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[1]Present address: Oppdrett Services (Canada) Ltd., 646 Fairville Blvd., Suite 202, Saint John, N.B. E2M 4Y7.

surpluses recently, and both Scotland and Norway have been plagued with furunculosis outbreaks. Losses have caused numerous hatchery bankruptcies in Norway. Our problems seem minimal by comparison.

Our low grilse rate is a big advantage. We can produce an 8 pound fish in 17 months. The Saint John River strain that is the basis for our aquaculture stocks is considered one of the best in the world. Supply and demand for smolts are almost balanced. Our location is extremely advantageous, as we are only an eight hour drive from major U.S. markets and one hour by air from the most populous region in Canada, making us very competitive with Europe. No wonder aquaculture has grown to be an $70-$80 million industry, bigger than the $54 million New Brunswick dairy industry.

THE FUTURE

Areas of concern for the future are problems in offshore technology and the need for new developments in offshore cages, the effectiveness of vaccines, and a better understanding of genetics, biotechnology, and the carrying capacity of the marine environment.

SGRP PRIORITIES AND OPPORTUNITIES

1. SGRP as a Primary Breeder.

Industry is the beneficiary of work done by the Department of Fisheries and Oceans (DFO) and the SGRP. The SGRP has developed one of the best salmon farm stocks in the world. Despite the difficulty in explaining the function of a breeding program, most farmers now understand the basics of what takes place, and contributed willingly this year to the support of the SGRP through the surcharge levied by the New Brunswick Salmon Growers Association (NBSGA). The SGRP should continue with broodstock work and examine the feasibility of establishing a gene bank for back-up in case of severe losses due to disease or other disasters.

2. Development of a High-S1-Low-Grilsing Strain.

S1 smolts usually demonstrate a lower grilsing rate in sea cages than S2's and are more cost effective to the hatchery operator. Sea Farm can only produce half and half, in spite of heat pumps and other treatments. There is no room for a grilse strain in the industry. With profit margins shrinking all the

time, it is imperative that market fish be no less than 9-11 pounds.

3. Vaccine and Disease Experiments.

There is still a lot of vibriosis around this year, in spite of the use of vaccines. We need to know what works and what doesn't. More independent, third-party investigation into the claims of the vaccine producers is needed. Sea Farm has had good results with a spray vaccination program undertaken on 100,000 market fish this year in Bliss Harbour - there has been no vibrio outbreak to date. This was a first trial, and it is hoped that the results are repeatable. It cost $25,000, but Sea Farm would have had to spend double that amount treating sick fish.

4. Training of Hatchery Technicians and Biologists.

There is a real shortage of trained cage workers. And, as great a shortage of cage workers as there is, there is an even greater shortage of trained hatchery and farm managers. A person must be something of a jack of all trades to function effectively in a hatchery or farm environment, and it is difficult to find people with the right combinations of knowledge and experience.

5. Visiting Lecturers.

Lectures, workshops and seminars, such as the Hatchery Management Workshop the SGRP hosted in July, 1989, are very important, and must continue to be a large part of what the SGRP does. The sharing of expertise and experiences are crucial.

6. Fall Parr Transfer.

Sea Farm engaged in an inadvertent fall parr transfer trial in 1988, and results were so promising that we plan to repeat the trial this year[2]. Sea Farm found itself with 200,000 surplus parr that couldn't be sold, so the decision was made to do some experimenting. The parr were divided among three farms, where losses due to transportation problems and strong currents ranged from 10 to 60 percent. Despite the losses, Sea Farm considered the survival good, since the fish would have been destroyed otherwise. Growth rates were much better than expected, with the parr growing

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[2] The 1989 transfer of fall parr was extremely successful, with only 1-5 percent mortality.

from 100 gm in the fall to 220 gm in the spring. Projected forward, growth rates for parr transferred in the fall to cage sites would produce a 10 pound fish in 19 months. This opens up whole new marketing possibilities. Now, fish can be ready for marketing in June, when product supply is the lowest and, consequently, price is the highest. Market share is increased, and the time when fish are available during the year is greater.

There are several explanations for the success of the trial. Temperature shock was minimal, due to the hatchery and ocean being roughly the same temperature at this time of the year. The parr were 100 - 125 gm in size, and bigger parr such as these are more apt to survive. The fish that died in larger numbers were parr that were still distinctly at the parr stage, and didn't appear to be entering the transformation to the smolt stage. This means that there is an easy visual check to determine the survivability of parr that are transferred in the fall: distinct parr marks.

Factors to be considered in determining the feasibility of transferring parr in the fall are:

1. Economics

   - poor growth initially
   - rearing expenses for 19 months vs. 17 months
   - grilse rate as yet unknown
   - initial losses

2. Fish health

   - survival
   - when to vaccinate

All in all, this is an exciting development in the industry, and other farms have expressed interest in pursuing trials.

7. Demonstration Hatchery to Test Equipment.

The SGRP could act as a demonstration operation for hatcheries, paralleling what the Atlantic Salmon Demonstration and Development Farm (ASDDF) does for sea cage technology.

8. Dry vs. Moist Hatchery Diet Evaluation.

The evaluation of diets and feeding regimes is an area that the SGRP can pursue.

9. Extension Work.

The SGRP must do more extension work in order to sell itself to the industry. Representatives of the SGRP should visit each farm and do an education job in layman's language. An extension program, similar to that in the Department of Agriculture, would be good public relations.

PLOIDY AND STERILITY IN SALMONIDS

Dr. Tillmann J. Benfey, Department of Biology, University of New Brunswick, Fredericton, N.B., E3B 6E1.

Maturing salmonids go through a number of physiological changes that, although of obvious importance for normal reproduction, are detrimental to the quality of fish destined for human consumption. For instance, there is a decline in flesh quality and colour, as lipids, proteins, carbohydrates and pigments are removed from the muscle, and the silvery external appearance is lost. Maturing salmonids are also more susceptible to disease and often have a high rate of mortality, sometimes reaching 100 percent in Pacific salmon. There is thus a need for sterilization or at least delayed maturation in species that mature prior to harvest in an aquaculture setting. There is also interest in releasing sterile fish into the environment to grow to a larger size (for the sports fishery), and to limit the number returning to spawn (for enhancement of the open-sea commercial fishery), or to prevent naturalization of exotic species.

There are several methods available to sterilize salmonids (Donaldson and Hunter, 1982), the most practical of which are the use of steroids or the induction of triploidy (Donaldson and Benfey, 1987). Triploid fish have three sets of chromosomes rather than the normal (diploid) number of two. They are generally produced by applying thermal or pressure shocks to eggs shortly after fertilization, thereby preventing extrusion of the haploid second polar body (Figure 1). The actual treatments that are most effective appear to be somewhat dependent upon the species; details can be found elsewhere for rainbow trout (Bye and Lincoln, 1986), Pacific salmon (Benfey and Donaldson, 1988), and Atlantic salmon (Johnstone, 1989). Effective treatments for producing triploid salmonids are generally on the order of 10 to 20 minutes at 26° to 28°C (heat shock) or 4 to 5 minutes at 9,000 to 10,000 p.s.i. (pressure shock), applied 15 to 25 minutes after fertilization and incubation at 10°C. The duration of the shock in these cases is inversely proportional to its magnitude. Certain anaesthetics have also been used to induce triploidy in salmonids (Shelton et al., 1986); Johnstone et al., 1989). Alternatively, tetraploids can be produced (Figure 1), raised to maturity, and crossed with diploids to yield all-triploid progeny (Chourrout et al., 1986; Chourrout and Nakayama, 1987).

As triploid fish are identical to diploids in external appearance, they can only be distinguished from diploids prior to

maturity by various cytological means. These include counting chromosomes or nucleolar organizing regions in appropriately prepared chromosome spreads, measuring cellular dimensions by microscopy or automated particle sizing, or measuring DNA content by microspectrophotometry or flow cytometry. Further references and a discussion of the advantages of these various techniques can be found in Benfey and Donaldson (1988).

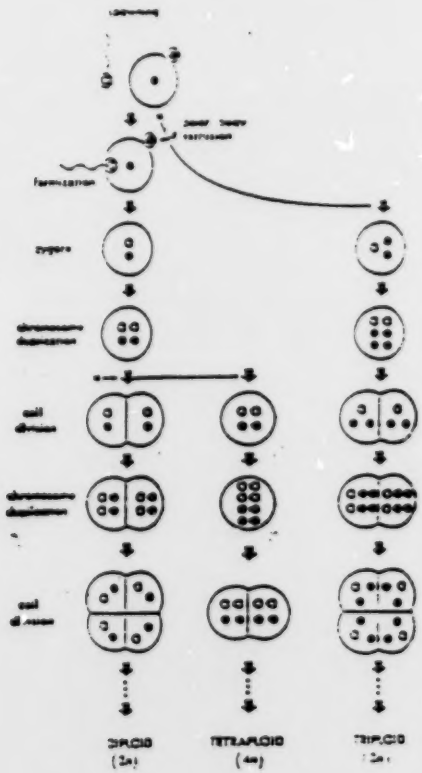

Figure 1. Timing of shocks (*) for producing triploids and tetraploids. Closed circles and stars represent chromosome sets of maternal and paternal origin, respectively. Reproduced from Donaldson and Benfey (1987), page 115.

Triploids are sterile because their pregametic cells (oogonia in females and spermatogonia in males) cannot go through normal meiotic reduction. The bulk of these cells fail to complete

meiosis, and those few that do (only in males) are aneuploid (Benfey et al., 1986). However, such aneuploid spermatozoa are fully functional and will activate development in normal eggs, thereby generating aneuploid progeny (Ueda et al., 1987) that die prior to yolk absorption (Lincoln and Scott, 1984). This is an important point to consider if triploid males escape or are intentionally released into the environment, where they can be expected to interact with normal females.

Testes are typically much larger than ovaries when cells enter meiosis, because of the enormous difference between males and females in the number of pregametic cells produced. As a result, testes of triploid fish become quite large, whereas ovaries remain very small. This leads to a pronounced physiological difference between triploid males and females, because gonadal steroidogenic cells associated with maturation are fully developed and functional in males but absent in females. As a consequence, triploid males go through all the endocrine and physiological changes associated with normal maturation, whereas triploid females remain endocrinologically and physiologically immature (Lincoln and Scott, 1984; Benfey et al., 1989). Although triploid males are sterile in the true genetic sense of the word, only triploid females have the characteristics of sterile fish desired for aquaculture and stock enhancement. All-female stocks can be made quite easily by using androgens to masculinize genotypic females, and then using their sperm to fertilize normal eggs (Donaldson and Benfey, 1987). Such a technique has been used to produce all-female triploid rainbow trout (Bye and Lincoln, 1986), Pacific salmon (Piferrer et al., 1988), and Atlantic salmon (Johnstone, 1989).

There is, unfortunately, little reliable data on the performance of triploid salmonids in aquaculture. It can safely be said that results have been better with pilot-scale trials than when done on a commercial-scale. Triploids appear to have normal survival and growth rates when reared under optimal conditions, but are less tolerant of sub-optimal rearing conditions. This has certainly been my experience with Pacific salmon, and I believe that of others working with Atlantic salmon. Triploid rainbow trout perform well in a commercial aquaculture setting, and are now used extensively for this purpose in the U.K. (Bye and Lincoln, 1986). This may be a reflection of greater domestication than an inherent difference between trout and salmon.

It must be emphasized that triploidy does not of itself confer any growth advantage to fish and therefore should only be considered in aquaculture as a tool to prevent early maturation.

Tripliody would be useful with species where both sexes may mature prior to harvest (e.g., rainbow trout and some stocks of coho and Atlantic salmon). In species where it is only the males that generally mature prior to harvest (e.g., chinook salmon and some stocks of coho and Atlantic salmon), the production of all-females is sufficient to alleviate maturation-related problems. In any case, the production of all-females must always be a first step if triploids are to be used for aquaculture.

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QUESTIONS

John Anderson: There are deleterious effects from interactions between wild and aquacultural stocks, causing an ever-increasing need for sterile fish.

Tillmann Benfey: I agree. Cage fish inevitably will and do escape. A method must be found to prevent cage fish from crossing with wild stocks.

John Bailey: Shouldn't we be careful not to use sterile males, for reasons you mentioned?

Tillmann Benfey: Yes. Sterile females are more suitable for aquaculture anyway, due to their failure to mature sexually.

NUCLEAR CATARACT FREQUENCY IN LAKE TROUT RECOVERED FROM LAKE ONTARIO IN 1988

Harold L. Kincaid, U.S. Fish and Wildlife Service, National Fishery Research and Development Laboratory, R.D. #4, Box 63, Wellsboro, Pennsylvania, 16901.

Cataracts in hatchery reared lake trout (Salvelinus namaycush) have been attributed to several causes: nutritional deficiencies (Hughes 1985), mechanical trauma (Riis et al. 1974), ultraviolet light (Allison 1963; Steucke et al. 1968), dissolved gas supersaturation (Poston et al. 1973), and temperature shock (Poston et al. 1973). Extensive studies of nutritional cataracts from 1973 to 1985 identified six specific nutritional deficiencies that cause the disorder (Hughes 1985). Nuclear cataracts develop in the inner nuclear tissue of the lens. Inheritance studies in lake trout showed a higher incidence of nuclear cataracts in families from parents with cataract than in those from normal parents, but did not show simple mendelian inheritance (Kincaid 1987). Other studies have also suggested a genetic basis for cataract (Allison 1963, Steucke et al. 1968, and Orme and Lemm 1974).

Cataracts appear as a cloudy or opaque area in the lens. Large cataracts can be seen easily with the naked eye, but supplemental light and magnification are needed to detect small cataracts. We were able to detect cataracts as small as 0.01 mm under field conditions using a 1.7x power illuminated magnifying lamp.

Examination of four 1985-year-class strains as yearlings at the Allegheny (Pennsylvania) National Fish Hatchery showed frequencies of nuclear cataracts ranging from 4 to 33 percent. More than 95 percent of cataracts found covered less than 1 percent of the lens cross-sectional area. The cataracts were not detected by hatchery personnel because they were small and the affected fish did not show behavioural changes in production raceways. A 1986 survey of lake trout reared at federal and state hatcheries showed cataract frequencies of 0 to 64 percent. The high frequency in hatchery-produced fish raised the question of the effect of the cataracts on the growth and survival of affected fish that were stocking in the Great Lakes. Studies to measure the effect of cataracts on the growth and survival of hatchery-reared lake trout after stocking in Lake Ontario were conducted in 1987.

METHODS

Samples of 300 fish from each strain produced at the Allegheny Hatchery, from the 1985, 1986, and 1987 year-classes, were examined as yearlings and cataract frequency was determined 6 weeks before the fish were stocked in Lake Ontario. Each stocking group (strain of a given year-class) was tagged with coded-wire nose tags at the fall fingerling stage to provide identification of strain and year-class at recapture. Lake trout, captured by bottom trawling in Lake Ontario in 1987 and 1988, were examined for nuclear cataract and the frequency in each stocking group was determined. The effect of cataract on fish survival was assumed to be the difference in cataract frequency at stocking and at recovery. The effect of the cataract on growth rate was measured as the difference in attained size between the nuclear-cataract and normal-eyed fractions of each stocking group at recovery.

Nuclear cataract and growth rate

A total of 2829 lake trout were recovered from Lake Ontario during the 1988 assessment season. Fish were captured from strains stocked between 1975 and 1988. The mean frequency of nuclear cataracts was 29 percent.

Comparison of the cataract and non-cataract fractions within individual stocking groups showed that the mean weight of the cataract fraction was larger in 70 percent of the groups. This trend was uniformly present in each strain. A comparison of size differences between normal-eyed, unilateral cataract (cataract in one eye) and bilateral cataract (cataract in both eyes) fractions, showed the cataract phenotype to be associated with higher weights (Table 1). Comparison of mean weights of the three eye phenotypes showed that, in 23 percent of the stocking groups, normal fish weighed significantly less than fish with unilateral cataracts. Fish with bilateral cataracts tended to be intermediate in weight. Body length comparisons showed the same patterns among the cataract and normal fractions. These findings suggested that faster-growing fish were the most susceptible to cataract development in the lake. The presence of small cataracts in one eye did not appear to interfere with normal feeding.

Nuclear cataract and survival

The frequency of nuclear cataracts ranged from 11 to 54 percent in the year-classes from 1980 to 1987. Regression of year-class cataract frequency on years of lake residency ($r=-2.86$, $N=6$,

$P<0.05$) was significant, showing that the frequency of nuclear cataracts decreased with increasing time of lake residency. Comparison of cataract frequencies at stocking and after 2 months of lake residency showed a reduction in the 1987 assessment, but no effect in the 1988 assessment.

Nuclear cataract frequencies at recapture in fish of the 1984 to 1987 year classes showed differences associated with strains and year-classes. Cataract frequency in recaptured fish of the Lake Superior strain in 1984-1987 ranged from 3.7 percent to 17.3 percent (mean = 5.7 percent), perhaps reflecting large year-to-year environmental variability (Table 2). Differences in cumulative cataract frequency after 2 years in strains stocked in 1985 (4.7 percent to 31.1 percent) and 1986 (12.3 percent to 45.5 percent) indicated genetic variability. The proportion of fish with nuclear cataract at stocking and recovery in both year-classes was highest in the Seneca and lowest in the Superior strain. Nuclear cataract frequencies at recapture after one year in the lake were reduced in the 1987 assessment, but unchanged in the 1988 assessment. One group, 1986 Jenny Lake, had significantly fewer nuclear cataracts at recovery (Table 2).

Stocking yearlings vs fall fingerlings

The season and type of fish stocked (fall fingerlings versus spring yearlings) strongly influenced cataract frequency in fish recovered from the lake. Returns from fall plants of fingerlings consistently had fewer cataracts than spring plants of yearlings of the same strain and year-class (Table 2). In 1987, three groups of fish produced from the same strain and year-class were stocked both as fall fingerlings and as spring yearlings. The nuclear cataract frequency was lower in fish from the fingerling plants than in fish from the corresponding yearling plants at both initial stocking and recovery. When cataract frequency data were pooled across these groups, fingerlings had only 24 percent as many cataracts as the paired yearling groups.

Survival of fish with nuclear cataracts relative to normal-eyed fish in the 1985 strains stocked as yearlings ranged from 45 to 59 percent after 14 months in Lake Ontario. Relative survival of the 1986 strains ranged from 17 to 192 percent after 2 months in the lake and from 66 to 197 percent after 14 months (Table 2). Estimates of relative survival of fish with the nuclear cataract phenotype stocked as fall fingerlings was 11 percent in the 1985 stocking after 22 months and 105 percent after 34 months.

DISCUSSION

Observations of nuclear cataract in hatchery-reared lake trout before and after stocking in Lake Ontario indicated that growth and survival characteristics in fish with the cataract phenotype differed from those in normal fish during lake residency. In fish from the 1985 to 1987 year-classes reared at the Allegheny Hatchery, the frequency of nuclear cataracts at stocking was highest in the Seneca strain and lowest in the Superior strain of all three year-classes. This consistent pattern of cataract frequency among strains led me to conclude that lake trout inherit a genetic predisposition for nuclear cataract development, but that actual development of the cataract is triggered by a variety of different stresses in the rearing environment.

Nuclear cataract frequencies in the separate groups at stocking and recovery show a pattern suggesting that survival is lower in fish with the cataract phenotype than in fish with normal eyes. Of the fish found to have nuclear cataracts before stocking, 95 percent had small stage-one cataracts covering less than 0.5 percent of lens cross section. Fish recovered from the lake had only the small stage-one cataracts. Hatchery fish, found to have nuclear cataracts in 1985 and observed in the hatchery over the past 3 years, have shown no indication of regression in cataract size. Therefore fish stocked in Lake Ontario with cataracts larger than stage one were lost. The loss of fish with large cataracts, combined with the finding of no difference in weight between cataract and non-cataract fish in the 1987 data, indicates that stage-one cataracts did not affect fish growth. However, when cataracts increased in size beyond stage one, the fish did not survive. The fact that there was no suggestion of reduced weight as cataract size increased implies that the fish were lost soon after the cataract reached the critical size, probably to predation. The 1988 results showed that fish with unilateral cataracts were consistently heavier than those with bilateral cataracts and that fish with either unilateral or bilateral cataracts were heavier than normal fish. This pattern suggests that faster-growing fish were more susceptible to cataract development, and supports the conclusion that, as cataracts develop beyond a critical size, the fish are quickly lost from the fishery. The fact that fish with bilateral cataracts weighed less than those with unilateral cataracts suggests that the presence of even small cataracts in both eyes resulted in reduced growth.

The increase in cataract frequency in eight of nine groups stocked as yearlings in 1987 and 1988, suggests that new cataracts

developed in normal fish between the two sampling periods. The 1987 data showed a general pattern of lower survival in fish with the cataract phenotype in the six 1985 and 1986 groups (Table 2). This pattern was completely reversed in the 1988 data, when cataract occurrence increased in all six groups. Since cataracts do not regress, this reversal must be the result of new cataract development in normal fish. Cataract frequencies of 8.3 to 23.1 percent in fish recovered from the 1982 to 1984 year-classes also suggested that additional cataracts developed after the fish were stocked. In light of the expected lower survival rate for fish with cataracts, it is difficult to understand the presence of high cataract frequencies in fish that have been in the lake for up to 6 years, unless cataracts develop as the fish age during lake residency. Reduced survival of the cataract phenotype was inferred from differences in the frequency observed in a group measured before stocking and at recovery. If additional cataracts develop after stocking, the approach used here overestimates actual survival.

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Table 1. Attained weight of eye type fractions in stocking groups recovered from Lake Ontario during the 1988 trawling season. All fish were stocked as yearlings.

Year Class	Eye Type	Strain											
		Superior			Seneca			Ontario			Jenny		
		N	Mean[a/]	SD	N	Mean[a/]	SD	N	Mean[a/]	SD	N	Mean	SD
1984	Normal	21	1227	515	33	1147	453	11	973	216			
	Unilateral	4	1450	610	8	1402	516	1	1093	---			
	Bilateral	2	1338	165	7	1288	374						
1985	Normal	59	585	242	111	649	270	15	474[a]	175			
	Unilateral	4	770	333	29	618	196	4	641[ab]	168			
	Bilateral				14	689	238	6	664[b]	203			
1986	Normal	292	219	95	72	268	116				42	239	107
	Unilateral	23	239	99	27	279	143				14	295	114
	Bilateral	14	270	108	36	256	102				10	229	90
1987	Normal	52	42[a]	18	90	43[a]	19	2	42	18	19	35	13
	Unilateral	7	57[b]	8	64	51[b]	19						
	Bilateral	14	46[ab]	14	96	50[ab]	19	1	39	---	5	44	9

a/ Significant differences ($P < 0.05$) between eye type fractions of the same strain are identified by different letters.

Table 2. Nuclear cataract frequency and survival of lake trout recovered from Lake Ontario in 1987 and 1988, by stocking group.

Stocking Group (Year and Strain)	Initial Cataract (%)[a]	1987 Recovery				1988 Recovery				Cumulative	
		Period in Lake (months)	No. Fish	Cataract (%)	S_r[b] (%)	Period in Lake (months)	No. Fish	Cataract (%)	S_r[b] (%)	No. Fish	Cataract (%)
Yearling Stocking:											
1984											
Ontario	---	26	45	10.9	---	38	12	8.3	---	57	10.5
Seneca	---	26	82	23.2	---	38	58	31.3	---	140	24.3
Superior	---	26	81	17.3	---	38	27	22.2	---	108	18.5
1985											
Ontario	31.7	14	37	24.3	0.45	26	24	41.7	1.54	61	31.1
Seneca	34.6	14	172	23.8	0.59	26	130	28.5	0.75	302	25.8
Superior	7.3	14	109	3.7	0.48	26	63	6.4	0.86	172	4.7
1986											
Jenny Lake	46.3	2	47	12.8*	0.17	14	66	36.4	0.66	113	26.5*
Seneca	51.0	2	78	37.2	0.57	14	111	51.4	1.01	189	45.5
Superior	6.7	2	58	12.1	1.92	14	267	12.4	1.97	325	12.3
1987											
Seneca	64.0			---		2	235	65.5	1.07	235	65.5
Superior	25.3			---		2	73	28.8	1.19	73	28.8
Fingerling Stocking:											
1985											
Seneca	34.6	22	19	5.3*	0.11	34	28	35.7	1.05	47	23.4
1986											
Seneca	51.0	10	7	0.0	---	22	27	33.3	0.48	34	26.5
Superior	6.7	10	22	4.6	0.67	22	62	6.5	0.97	94	5.3
1987											
Jenny Lake	16.7										
Seneca	64.0					10	24	20.8	1.32	24	20.8
						10	17	47.1	0.50	17	47.1

a/ Frequency of nuclear cataract in stocking groups 30 to 45 days before stocking in Lake Ontario as yearlings.

b/ Relative survival (Ricker 1975)

QUESTIONS

Richard Saunders: During salinity challenges and in connection with osmoregulatory problems, we see cataracts develop. What type might this be?

Harold Kincaid: I can't be sure, because cataracts can result from many different causes. Six different types of nutritional cataracts have been studied. Also, chemical pollutants have been shown to cause cataracts. An increasing occurrence of cataracts is reported in Atlantic salmon off the coast of Scotland that is suspected to result from exposure to the chemical Nuvan, used in commercial cage culture operations. In the case of salinity challenges specifically, I would suspect that differential rates of salt uptake by the nuclear and cortex tissues of the lens during the challenge could cause stresses leading to development of lesions (cataracts) as the fish adjusts to the higher salinity level.

Roger Doyle: Are there any inbreeding or dominance effects?

Harold Kincaid: No work has been done on this question. We have looked at different strains in hatchery populations over the past four years and consistently find Seneca strain fish with higher cataract levels than Superior strain. The specific cause for genetic predisposition to the development of cataracts is not known.

Gerry Friars: What is the incidence of cataracts in natural fish in Lake Superior?

Harold Kincaid: This is unknown. We work with fish recovered from Lake Ontario, where all fish are stocked from hatchery production as either fall fingerlings or spring yearlings. Cataract frequencies as high as 40 percent were found in some groups that have resided in the lake for over five years.

Gil Farmer: Is nutrition a factor?

Harold Kincaid: Maybe. Nutritional deficiencies do cause cataracts; therefore, nutrition can't be ruled out. Nutrition is probably not the prime factor, because the same diet is used to produce fish at different hatcheries throughout the country and we still see large differences in cataract frequencies between hatcheries, among strains at the same hatchery and among lots of the same strain produced at different hatcheries.

Roger Doyle: Instead of there being a physiological correlation between growth and cataract development, could it be that the fish that have cataracts must grow faster in order to survive?

Harold Kincaid: It seems logical that cataracts must inhibit growth in some way, via inhibiting feeding or the ability to escape predation. Laboratory studies have tended to find that cataracts develop in the faster growing fish, suggesting that the faster growth rate itself contributes to cataract development. Comparisons of fish with bilateral versus unilateral cataract showed that, in fish from the same lot, fish with bilateral cataract were smaller. This showed that reduced vision did retard growth rate.

John Bailey: The fish are protected in an aquaculture situation, so could we screen for cataract fish in order to pick up on faster growth rate and utilize this characteristic?

Harold Kincaid: Cataracts were larger in hatchery than in wild fish. It is assumed that the small cataracts have no effect on vision initially, but as the cataracts grew, vision was more seriously impaired and higher mortalities resulted. One would expect the effect of reduced vision to be far more serious in the natural environment. I doubt that selection for cataract to obtain a correlated response in growth rate would be beneficial in either the hatchery or cage environments.

Richard Saunders: Are there any studies on the effects of light on the development of cataracts? For example, lake trout are a deepwater species being reared in shallow conditions - has any work been done in this area?

Harold Kincaid: Yes. In a 1968 paper by Steucke et al, lake trout reared in outdoor tanks had an incidence of 80 percent after one year, whereas the occurrence in companion indoor-reared fish was trivial. The lots were then split and half the inside group were moved outside. Likewise, the inside group was split with half remaining inside and half moved outside. After one year, fish moved outside from inside tanks had as many cataracts as the group that had been outside over the two year test period. Steucke et al concluded that exposure to UV light was a major factor in cataract development.

John Anderson: So the simplest explanation may be the light, not a general predisposition at all.

Harold Kincaid: Exposure to UV light is certainly one cause of cataracts, but light alone does not explain cataract development in fish that reside in deep water, such as in the Great Lakes. Also, the fact that different strains of fish reared side-by-side under the same rearing conditions have different cataract frequencies show that strains have differential susceptibility to cataract.

Fiona O'Flynn: In the initial stages of development, did the hatchery fish have an advantage over the fish outdoors?

Harold Kincaid: There were no apparent differences in attained weight or survival in fish reared in inside and outside raceways during our studies. All fish were reared inside the hatchery building with artificial light and natural photoperiod until they were 15 months old. The fish were then moved outside and half were put in tanks under a Hansen cover that eliminated all exposure to UV light. Continuous subdued light and a natural photoperiod was provided. The other half were put in identical tanks located in the open and exposed to direct sunlight throughout the day.

CRYOPRESERVATION OF SALMONID SPERM

Richard Gallant, Prince Edward Island Department of Fisheries and Aquaculture, Box 2000, Charlottetown, P.E.I., C1A 7N8

Salmonids undergo yearly reproductive cycles, spawning in late October or November. Semen from males contains billions of sperm cells bathed in seminal plasma. Reported concentrations of sperm for salmonids range from 9-18 x 10^9 sperm/ml semen. Because the spawning season is short in salmonids, new techniques are required to preserve genetic material for use at other times. One objective of this thesis was to cryopreserve semen that would be used later to fertilize eggs. This presentation will focus on the aspects of cryopreservation and describe techniques used in our laboratory to assess the viability of frozen-thawed semen.

Cryopreservation is the low temperature storage of cells. Liquid nitrogen is commonly used to cryopreserve cells at low temperatures (-196°C) for extended periods of time. Cryopreservation originated in the 1770's in the livestock industry. Cryopreservation of fish sperm began in the 1950's and 1960's, when researchers froze sperm from herring and Atlantic cod. Salmonid sperm cryopreservation came into focus in the 1970's, with the expansion ofaquaculture and the increased awareness of the potential uses of cryopreserved products in aquaculture.

Cryopreservation of salmonid sperm would be beneficial to the aquaculture industry for the following reasons:

1. The number of male fish in hatcheries could be reduced, resulting in lower costs for feed and care.

2. Genetic material could be shipped world-wide.

3. Genetic material with desirable qualities, such as disease resistance, could be available after the death of the individual.

4. A broad-based gene pool could be set up and conserved for the future.

The procedure for semen collection involves anaesthetizing the fish, wiping it dry (so as not to contaminate the milt with other fluids such as water) and stripping the milt by abdominal massage.

In order to cryopreserve salmonid sperm, two agents must be used - an extender and a cryoprotectant. An extender is a solution of salts that is isotonic with sperm cells and has a good buffering capacity. The extender must not activate sperm cells and it may contain organic compounds, such as proteins, to protect cell membranes. A cryoprotectant functions to prevent ice crystal formation in the cell by binding to electrolytes to prevent their concentration in and around the cell. Examples of cryoprotectants include glycerol, dimethyl sulfoxide, methanol and dimethylacetamide. Milt is added to the extender solution and thoroughly mixed. The material is then drawn into 0.5 ml straws, sealed with a plug and stored in a programmable cell freezer. Alternative storage methods include: (1) pellets which are placed in depressions in dry ice and (2) straws immersed in liquid nitrogen vapour. Straws are stored in goblets and canes in liquid nitrogen.

There are inconsistencies in the literature regarding methods for assessing the viability of frozen-thawed salmonid sperm. The methods used in this study included motility observations, fluorometry, LDH leakage and the rate of successful fertilization in trial lots of eggs. Fluorometry shows much promise as a quick, inexpensive indicator of sperm viability. Motility observations are subjective by nature. Sperm counts vary widely from fish to fish and can affect fertilization. Steps in the preparation of sperm cells for LDH assay, such as centrifuging can damage sperm cells. Lastly, fertility results can be even more variable than motility, fluorometry or LDH determinations.

Motility is determined after semen is thawed by rating the percentage of motile cells and type of movement on a scale of 0 to 10. The semen is thawed in a water bath to prevent the formation of ice crystals on the outside of the straw. The sperm is activated with a NaCl solution at 4°C.

The fluorometric technique is based on the principle that a dye, ethidium bromide (EtBr), enters cells with disrupted membranes, binds to undenatured nucleic acids and induces an intense fluorescence. A digitonin solution is then used to rupture all sperm membranes in the sample to determine the proportion of cells with damaged membranes.

Enzyme leakage measurements from the cytoplasm of damaged cells has been useful in assessing sperm viability in chickens and turkeys. LDH concentration of the seminal plasma from rainbow trout was determined by centrifugation at 500g, followed by

dilution with 3.2 M ammonium sulfate and spectrophotometric assay.

A curve was established to determine sperm concentration by optical density. Semen was collected and pooled from several fish. Pooled semen was divided into several aliquots and diluted with 1.1 M sodium citrate to give a range of sperm concentrations, and an absorbence was recorded on a spectrophotometer for each sample. Sperm cell counts were done using a haemacytometer. Linear regression was used to predict concentration of sperm (in billions per millilitre of semen) by optical density. This regression equation was used to determine the concentration of sperm in each tube and lots of 300 eggs were fertilized with 200,000 sperm per egg. Eggs were incubated in Heath Incubators supplied with well water at 10°C.

In all experiments, dead eggs were removed periodically from incubators and cleared. The presence of an embryo upon clearing was recorded as a fertilized egg. Before hatching, live eyed eggs were counted for each treatment and the proportion of eggs fertilized was determined.

In conclusion, cryopreservation will be increasingly used for the preservation of genetic material. New methods to evaluate sperm viability, such as the development of better extenders, will enable researchers to avoid fertility testing. Finally, the use of programmable cell freezers will allow for consistent, repeatable results.

(The following paper[3], presented at the Aquaculture Association Conference in St. John's, Nfld in July, 1989, describes in greater detail several methods studied as part of a MSc. thesis to assess the viability of cryopreserved salmonid semen [Aquacult. Assoc. Can. Bull. 89-3, Sept. '89, pp. 37-39].)

IN VITRO METHODS TO ASSESS SALMONID SPERMATOZOAL VIABILITY AFTER STORAGE by R.K. Gallant and M.A. McNiven[4]

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[3] Minor edits by SGRP reviewers have been incorporated for inclusion in this technical report.

[4]Atlantic Veterinary College, University of P.E.I., Charlottetown, P.E.I., C1A 4P3

ABSTRACT. Three *in vitro* tests were evaluated to assess sperm viability of rainbow trout (Oncorhynchus mykiss). Motility, fluorometry, and lactate dehydrogenase leakage indicated significant relationships with the percentage of killed spermatozoa ($p<0.001$). The *in vitro* methods were significant in predicting fertility ($p<0.05$). There was no significant variation between or within experiments.

INTRODUCTION. To date, motility has been the lone *in vitro* method used to assess spermatozoal viability of salmonids. Its value in predicting fertility is questionable due to the subjectiveness of the method and the short duration of sperm motility upon activation.

Fluorometry, a technique which fluoresces the genetic material of damaged cells, has been used to evaluate semen quality of chicken, bull and human sperm (3, 4, 5). The fluorometric technique is based on the principle that a dye, ethidium bromide (EtBr), enters cells with disrupted membranes, binds to undenatured nucleic acids and induces an intense fluorescence. A digitonin solution is then used to rupture all sperm membranes in the sample to determine the proportion of cells with damaged membranes (4, 5).

Measurement of enzyme leakage from the cytoplasm of damaged cells has been used to assess sperm viability in fowl and turkey (6). Lactate dehydrogenase (LDH) is found in all tissues and catalyses the reversible reaction of L-lactate to pyruvate.

Fertility is the ultimate indicator of sperm viability. However, fertility trials are expensive to carry out, due to costs of eggs, incubation equipment, water pumping and the time factor until eggs reach the eyed stage.

The objective of this study was to evaluate the relationships between the proportion of killed sperm and motility, fluorometry, LDH leakage and fertility. The validity of using the *in vitro* methods to predict fertility was examined. Variation between and within experiments was evaluated.

METHODS. Semen was collected from adult rainbow trout by abdominal massage and pooled. To prepare killed sperm, a sample was sonicated for 90 seconds. Live and killed semen were mixed so that resulting proportions of killed semen were 0, 25, 50, 75 and 100 percent and the final volume of each tube was 3.0 ml. Tubes were randomized and stored at 4^0C.

Motilities were determined for each sample and rated on a scale of 0 to 10, depending on the percentage of motile cells and type of movement. Sperm were activated by 0.12 M NaCl at 4^{0}C.

Fluorescence measurements were taken using a Hewlett Packard MPF-66 Fluorescence Spectrophotometer with excitation wavelength of 360 nm and emission measured above 575 nm. A 10 ml sample was suspended in 3.990 ml of phosphate buffered saline solution, inverted for 60 seconds and fluorescence recorded. A 25 ml sample of digitonin solution was added, inverted for 60 seconds and fluorescence recorded.

LDH concentration of the seminal plasma was determined for each tube by centrifugation at 500g, dilution of seminal plasma with 3.2 M ammonium sulfate and assay spectrophotometrically (Coulter Electronics, Inc. Hialeah, FL).

A concentration curve was established to determine sperm concentration by optical density. Semen was collected from several fish, pooled, diluted with 1.1 M sodium citrate to give a range of sperm concentrations and an absorbence recorded using a spectrophotometer. Sperm cell counts were done using a haemacytometer. Linear regression was used to predict concentration of sperm (in billions per millilitre of semen) by optical density.

This regression equation was used to determine the concentration of sperm in each tube and lots of 300 eggs were fertilized with 200,000 sperm per egg. Eggs were incubated in Heath Incubators fed well water at 10^{0}C.

<u>In vitro</u> determinations of sperm viability and fertility were made three times for the live/killed proportions. A second sample of fresh semen was sonicated and the experiment was repeated.

Dead eggs were removed periodically from incubators and cleared in a solution of methanol, acetic acid and water (7). The presence of an embryo upon clearing was recorded as a fertilized egg. Before hatching, live eyed eggs were counted for each treatment and from this the proportion of eggs fertilized was determined.

Data were analyzed using linear regression analysis. Arcsin transformation was used to convert fluorometry and fertility results for the analysis. Indicator variables were used to test for variation between and within experiments.

RESULTS. Linear regression analysis of motility rating, percentage dead sperm by fluorometry, LDH concentration of the seminal plasma, and fertility on percentage killed sperm revealed significant relationships ($p<0.05$) with coefficients of determination (r^2) of 0.937, 0.992, 0.994 and 0.190, respectively. There was no significant variation between or within experiments. Table 1 presents the means and standard errors for motility, fluorometry, LDH concentration, and fertility using semen diluted in five proportions of live/killed sperm expressed as percentage killed.

The regression of sperm concentration on absorbence indicated a highly significant relationship ($p<0.001$) with r^2 values of 0.324, 0.142 and 0.158 respectively. There was no significant variation between or within experiments.

Table 1. Estimates of mean sperm viability using <u>in vitro</u> variables and fertility from live/killed proportions of rainbow trout semen (SEM for n=6).

Killed Sperm %	Motility 0-10	Fluorometry % Dead	LDH u/L	Fertility %
0	9.0(0.00)	1.7(0.18)	1.3(0.02)	20.5(3.69)
25	8.3(0.21)	32.6(0.42)	74.8(0.49)	52.7(5.10)
50	4.8(0.17)	56.6(0.38)	142.2(1.40)	54.6(4.77)
75	4.0(0.00)	78.6(0.32)	204.3(1.66)	43.8(3.82)
100	0.0(0.00)	97.9(0.38)	263.3(5.39)	0.1(0.18)

DISCUSSION. Fluorometry gives a good indication of the percentage of dead cells in a sample. Fluorometry was highly correlated with increased proportions of killed sperm. The fluorometry method is straightforward, rapid and requires only a small volume of semen. We recommend this method as an <u>in vitro</u> test of sperm viability for salmonids. Our findings are supported by Bilgili et al (8) who observed a high accuracy in estimating the predicted percentages of dead chicken sperm in prepared mixtures by fluorometry.

LDH leakage is a common diagnostic tool for <u>in vitro</u> diagnosis of human disorders such as myocardial infarction and pernicious anaemia. LDH isoenzymes have been isolated from the testes and sperm of human and rabbit (9) but not from fish sperm. The highly significant relationship between LDH concentration and increased proportions of killed sperm suggested this method might be a valuable aid to assess sperm viability. However, the volume of

semen required to determine LDH concentration is substantially greater than the volume required for fluorometry. The collection of seminal plasma takes time and there may be damaging effects during centrifugation. Also, LDH activity may vary from fish to fish and during the reproductive cycle.

Motility is an accurate parameter to assess the viability of sperm cells but requires considerable experience and may vary between individuals. The conditions under which motility ratings are made may vary between laboratories. Motility ratings were performed at 4^{0}C to assure against temperature shock to sperm by keeping materials in contact with the cells at the same temperature and reducing the heat of the microscope. The temperature of activation can have a drastic effect on motility. Lindroth (10) studied motility of brown trout (Salmo trutta) sperm and found that sperm activated at 20^{0}C, 10^{0}C and 5^{0}C had motility duration of 35, 60 and 90 seconds respectively. There is difficulty performing motility ratings on sperm with different concentrations and when dilution of cells for activation is not consistent.

The fertility results are more variable than motility, fluorometry or LDH determinations. A critical ratio of 200,000 sperm/egg was proposed by Moccia and Munkittrick (11) to maintain high fertility. The number of eggs per lot was over-estimated in this experiment to about 400, thus reducing the ratio to 150,000 sperm/egg. Limiting the number of sperm to a minimum served to increase the sensitivity of the trials, but may have added variation to the results. Using minute volumes of semen to fertilize eggs made it difficult to distribute semen evenly over all eggs. A sperm diluent might help in the fertilization process.

The significant results using fluorometry and enzyme leakage to predict fertility are supported by Bilgili and Renden (3) who reported significant relationships between percentage dead sperm and fertility and enzyme leakage with increased percentage dead cells for chicken semen. In conclusion, the value of using <u>in vitro</u> methods such as motility, fluorometry and enzyme leakage to predict fertility is warranted.

NOTES: (1) Dept. of Health Management, AVC, UPEI, Charlottetown, P.E.I., C1A 4P3. (2) We thank D. Steele and H. MacMillan for technical support and M. Hambrook and W. VanToever for broodstock use. (3) Bilgili, S.F., Renden, J.A., 1984. Poultry Science 63:2275-2277. (4) Halangk, W., Bohnensack, R., 1982. Acta Biol. Med. Germ. 41:899-905. (5) Peterson, R.N., Silverstein, K., Freund, M., 1974. J. Reprod. Fert. 41:485-488. (6) Wischart,

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QUESTIONS

Gerry Friars: Crim has suggested that survival at the eyed stage only is not a good indicator of real fertility success.

Richard Gallant: We actually took observations at later stages of development, but didn't report them. There did not seem to be any effects on hatching or swim-up.

John Cornick: Heat is used to seal the straws. Can this affect viability?

Richard Gallant: It could. The use of a plastic plug to seal the straws is an alternate method, but it is not reliable. We discarded the first drop of semen from the straw before doing viability tests.

Roger Doyle: At Dalhousie we would like to have a field technique for cryopreservation, to expedite DNA extraction. Is there a technique for such a procedure?

Richard Gallant: Dr. Mary McNiven had a cell freezer consisting of a simple canister containing liquid NO_2. Liquid NO_2 lowers the temperature gradually. The whole unit is small and portable. Others have used dry ice to freeze semen in the field or immersion into liquid nitrogen

Sandy McGeachy: How did the fertility rates compare for salmon versus trout?

Richard Gallant: Conditions were different and the salmon eggs were overripe. The fertility rate for salmon was 25 percent and 75 percent for trout. Twenty-five percent is an acceptable rate for superior fish, but for general use in aquaculture, at least 80 percent would be required.

Tillmann Benfey: Is it possible to freeze eggs?

Richard Gallant: No one has been successful with fish yet. However, some success has been attained with other species. The salmonid egg is a very large cell to dehydrate and freeze.

Roger Doyle: Is freezing necessary in order to keep milt viable for short periods?

Richard Gallant: No. Milt kept in cold storage, at 4^0C, was still good after 5 weeks. After the fifth week, though, viability dropped to 80 percent.

Gil Farmer: What are the best storage conditions for keeping milt for a couple of days?

Richard Gallant: Collect the milt water-free. Store it in sufficiently large containers at a volume no greater than 6 ml per container. Maintain a low temperature, no higher than 4^0C.

Tillmann Benfey: Is 100 percent oxygen necessary?

Richard Gallant: Not for short storage periods of less than a few days, but oxygen will improve storage ability over air.

Gil Farmer: Is storage in an ordinary refrigerator adequate?

Richard Gallant: Yes, as long as it is stored in an open container, or at least opened frequently, because sperm use oxygen.

Gerry Friars: Is motility a poor criterion for evaluating males?

Richard Gallant: No, it's a good criterion, but not particularly good as a detailed indicator.

DISEASE HISTORY AND DISEASE CONTROL PROGRAMS OF THE SALMON GENETICS RESEARCH PROGRAM AT THE ATLANTIC SALMON FEDERATION, CHAMCOOK, N.B.

John W. Cornick, Fish Health Service Unit, Department of Fisheries and Oceans, P.O. Box 550, Halifax, N.S., B3J 2S7.

INTRODUCTION

The success of the SGRP at the Atlantic Salmon Federation depends very heavily on the absence of disease. The DFO Fish Health Service Unit (FHSU) in Halifax, N.S. has been involved in diagnosing fish diseases and recommending control programs at the facility since early in 1976. In this presentation I will give a brief history of diseases identified at the facility and discuss the various disease monitoring and control programs undertaken there.

DISEASE HISTORY

Table 1. Fish diseases isolated at the Atlantic Salmon Federation, 1976-89.

	Age				
Year	Fry	UYP*	1+	2+	Brood
76			-(3)		
77	B(1/1)**				
78		B(1/1)			V(1/1)
79		B(15/15)			V(1/1)
80	B(1/2)		-(1)		B,E(1/1)
81	-(4)		-(2)	E,I(1/2)	I(1/3)
82		-(2)	-(2)		V,E(2/3)
83	-(2)		-(1)	I(1)	B(1/1)
84	-(4)	F(1/6)	F(6/7)	F(2/3)	
85	-(1)		-(5)		-(2)
86		B(2/3)	B(3/3)	-(1)	B(2/2)
87	C(1/1)	-(1)	-(4)	-(1)	B(1/2)
88			-(2)	-(3)	B(1/2)
89	-		-(3)	-(2)	B(1/1)

*Under-yearling parr.
**Disease (No. of lots positive/no. of lots examined).
B-BKD by DFAT; I-IPN; V-Vibriosis; E-ERM; F-Furunculosis; C-Costiasis

The diseases identified by the FHSU in the various life stages of salmon at the facility since 1976 are listed in Table 1.

Furunculosis was identified in under-yearling parr (UYP), 1+ and 2+ fish in 1984, but was successfully eradicated by selective stock destruction. Infectious Pancreatic Necrosis (IPN) was identified in the carrier state in brood and 2+ fish in 1981, and in 2+ fish again in 1983, but has not been identified since. The Enteric Redmouth (ERM) pathogen was found in older fish (broodstock and 2+) between 1980 and 1982, but was not associated with any particular losses. Vibriosis was confined to the broodstock held in salt water in the broodstock facility, but has not been identified since 1982. Bacterial Kidney Disease (BKD) in the non-clinical form (no lesions) is the most consistently found disease. It is present in all year classes at one time or another, but most consistently in the broodstock. In the last 3 years it has not been found in the younger fish.

DISEASE MONITOR PROGRAMS

The diseases most likely to threaten the SGRP are furunculosis and BKD. They are most commonly introduced with the transfer of fish and eggs. BKD is carried within the egg and furunculosis on the outside surface. The transfer of furunculosis via eggs, therefore, is effectively controlled when eggs are properly surface disinfected. Vertical transmission of BKD, however, cannot be effectively controlled by treatment. Instead we rely on monitoring the disease in the broodstock and eliminating infected fish from the breeding program. All eggs entering the facility since 1986 have been screened for BKD under this program. Both these diseases are commonly transferred in live fish, but are effectively controlled under Regional Fish Health Policy testing, including a requirement to test for and eliminate all fish transferred to marine cages which might be infected with the carrier state of furunculosis. The various disease monitor programs carried out by the FHSU for the SGRP since 1976 include the following:

1. Fish Health Assessment, 1976-77.

In 1976-77 an assessment of the disease status of the facility was carried out using FHPR schedule II methodology. Results appear in Table 2.

In 1976 two year classes (1+ and uyp) were examined and no FHPR pathogens were detected. In 1977, however, 2/3 of the 1+ lots were positive for BKD by fluorescent antibody examination (FAT) of

kidney smears. This was the first identification of BKD by FAT at the facility. As a result, more extensive monitoring of the stocks in all tanks was carried out.

Table 2. Fish health assessment, Atlantic Salmon Federation, Chamcook, N.B., 1976-77.

Date	Age	No.	Results
December, 1976	1+	57	Negative
	UYP	57	Negative
August, 1977	1+	34	2/3 positive BKD(DFAT)*

*Direct fluorescent antibody test

2. Chamcook Lake Monitor for BKD, ERM and Furunculosis.

The question concerning the source of the BKD in 1977, ERM in 1980, and furunculosis in 1984 resulted in monitoring of wild stocks in Chamcook Lake, the water supply for the facility, in 1978 and 1984. Results are given in Table 3.

Table 3. Chamcook Lake monitor for bacterial kidney disease (BKD), enteric redmouth (ERM), and furunculosis disease agents.

Year	Exam Reason	Species	No.	Results
1978	source of BKD & ERM	lake trout	18	ERM neg. BKD pos.*
		land-locked salmon	12	ERM neg. BKD pos.*
1984	source of furunculosis	land-locked salmon	6	neg.
		sucker	31	neg.
		yellow perch	27	neg.
		eel	1	neg.

*subclinical BKD; kidney positive for BKD by direct fluorescent antibody test.

In 1978 lake trout and land-locked salmon were monitored, and although ERM was not detected, BKD was found in the kidneys of both species by FAT, indicating that the lake may have been a source of this agent. The source of the ERM, however, remains a mystery. In 1984, in an attempt to determine the source of the furunculosis outbreak at the facility, land-locked salmon, suckers, yellow perch, and eel were monitored from the lake, but furunculosis was not detected in the species tested. However, moderately high circulating antibody titers against this agent, detected in the blood of the suckers, suggested that these species may have come in contact with the pathogen sometime in the past.

3. Disease Monitor of Returning Broodstock.

Over the years moribund and dead broodstock from the brood facility have been examined for pathogens as the cause of death. The data appear in Table 4.

Table 4. Disease monitor of returning broodstock at the Atlantic Salmon Federation.

Year	Result
September, 1978	*Vibrio* sp.
August, 1982	*Vibrio anguillarum*
July, 1983	BKD*
1986-1989	BKD*

*BKD - subclinical; kidneys positve by direct fluorescent antibody test.

In 1988 a vibrio species was associated with some mortality. In 1982 *Vibrio anguillarum* was diagnosed as causing mortality. In 1983 and again between 1986 and 1989 BKD(DFAT) was detected in low numbers in the kidneys of some mortalities, but was not considered to be the cause of death. Because BKD can be vertically transmitted, it is important that brood fish be screened prior to stripping.

4. Furunculosis Carrier Tests.

As part of a co-operative DFO-N.B. Fisheries and Aquaculture disease control program, all fish transferred to marine cages from freshwater sites in the spring in N.B. are screened for the carrier state of furunculosis. Yearly tests since 1985 on stocks at this

facility going to sea cages (Table 5) have failed to detect carrier furunculosis in spite of the clinical disease outbreak in 1984. In this case, eradication appears to have been effective.

Table 5. Results of carrier tests* for furunculosis at Atlantic Salmon Federation.

Date	Stock	Results
February, 1985	parr	negative
April, 1985	smolt	negative
April, 1986	smolt	negative
March, 1987	smolt	negative
April, 1987	presmolt	negative
October, 1987	smolt	negative
March, 1989	smolt	negative

*Prednisolone acetate and heat stress test.

5. Marine Broodstock Monitor for BKD.

Since 1983, broodstock supplying eggs to this facility have been routinely monitored for BKD by FAT tests on kidney smears in lethally sampled fish or on reproductive fluids, in an attempt to avoid infected progeny. Results appear in Table 6.

Table 6. Marine broodstock monitor for bacterial kidney disease (BKD) for SGRP egg sources.

Year	Location	Results
1983	Fundy Aquaculture	BKD (clinical)
1985	ASF returns	negative
1986	Fundy Aquaculture	BKD(FAT)* - (9/58 milt 4/56 ovar. fl. pos.)
1988	ASF returns (A & B strains)	BKD(FAT)* in kidney in B strain, -light.
	Salmon Demonstration & Development Farm	negative
1989	Limekiln Bay (Strain A)	negative

*Direct fluorescent antibody test

In 1983 clinical BKD was detected in ASF brood being held at Fundy Aquaculture Ltd. marine cage site, so use of these brood was discontinued. Since that time, eggs have been stripped from stocks held at the Salmonid Demonstration and Development Farm. These fish have been monitored regularly and no BKD has been detected.

SUMMARY

The DFO Fish Health Service Unit in Halifax has diagnosed fish diseases and recommended control programs for the SGRP at the Atlantic Salmon Federation facility at Chamcook, N.B. since 1976.

The diseases encountered included clinical furunculosis in 1984 which was successfully eradicated; Infectious Pancreatic Necrosis (IPN) in the carrier state in broodstock and 2+ fish prior to 1983; subclinical enteric redmouth (ERM) in older fish (broodstock and 2+) between 1980 and 1982; and bacterial kidney disease (BKD) in the nonclinical state (no lesions) in all year classes at one time or another, but most consistently in broodstock.

Various disease programs have been carried out at the facility over the years, including (1) a general health assessment of stocks in 1976-77 when subclinical BKD was detected by fluorescent antibody test for the first time; (2) monitored wild fish in the Chamcook Lake water source in 1978 and 1984 as a possible reservoir for BKD, ERM and furunculosis infections which detected carrier state BKD only, indicating a possible source for that disease; (3) vibriosis and subclinical BKD were detected in broodstock examined since 1978; (4) routine monitoring of stock for carrier-state furunculosis since 1985 has failed to detect this disease and (5) since 1983 various marine broodstocks supplying eggs to the SGRP program have been monitored for BKD. Although clinical BKD was found in SGRP brood at Fundy Aquaculture in 1983 and this stock was abandoned, broodstocks monitored at other sites in subsequent years were clean.

QUESTIONS

Robert Cook: Was the broodstock with BKD you described primary broodstock?

John Cornick: Yes.

John Anderson: The broodstock monitoring for BKD is a long-standing record. Are there other long records?

John Cornick: Yes. Mactaquac has had long-range monitoring for furunculosis for some time. Enteric redmouth has been reported occasionally for some time, with the carrier state common in the wild. IPN is present in surface waters.

John Anderson: Would a UV disinfector be practical on a surface water supply system?

John Cornick: They are expensive, but very effective. However, with the methods that are in place now, furunculosis is basically under control, with the odd outbreak, so maybe the cost for a UV system could not be justified. It will not eliminate transmission in carrier infected fish.

Richard Saunders: Landlocked salmon broodstock has been collected from Chamcook Lake and the progeny subsequently released into the lake. Perhaps they brought the disease to the lake.

John Cornick: We tried to trace it, but were unsuccessful.

Floor: Can BKD be eliminated by disinfection at the egg stage?

John Cornick: No. It is the same for IPN. Disinfectants don't work because the pathogen is carried within the egg and not on the surface.

Roger Doyle: Is it impossible to remove BKD or just very difficult?

John Cornick: It's very difficult with the techniques currently available to us.

DISEASE MONITORING IN RELATION TO THE SALMON GENETICS RESEARCH PROGRAM

John O'Halloran, N.B. Department of Fisheries and Aquaculture, P.O. Box 5001, St. Stephen, N.B., E3L 2X4.

Dorland's medical dictionary defines disease as "any deviation from or interruption of the normal structure or function of any part, organ or system (or combination thereof) of the body that is manifested by a characteristic set of symptoms and signs and whose etiology, pathology and prognosis may be known or unknown."

Subclinical disease is where there are no readily observable signs of disease.

Disease resistance is a major factor to consider in selection in relation to nutritional, environmental and management aspects and specific disease pathogens. What good is a 5 percent increase in weight per salmon if you have a greater incidence of mortality due to a ubiquitous organism such as vibrio?

It is of extreme importance to keep records of levels of morbidity and mortality as well as the genetic basis of a particular stock. The cause of mortality and effectiveness of treatment is important in comparing production in different years.

In many terrestrial animal industries there are set rates of production, morbidity or mortality that determine when you should take corrective action in the face of disease. There are also expected results of your corrective actions.

The SGRP should have a set of steps to take when their fish are in trouble. Example: if fry mortalities start to rise in the month of June, at what level do you take corrective action? What testing is initiated on fish, water and feed when a problem occurs? What are the results of treatments or changes in management techniques? What preventive measures can be adapted next year to prevent a disease outbreak? If you do not have some of those parameters standardized and in 1990 you have a 2 percent decrease in fry mortalities, is it due to better management, nutrition or a genetically disease-resistant stock?

During the 1984 furunculosis outbreak at the Salmon Federation, mortalities in different family groups ranged from 2 percent to 82 percent. Later testing at the DFO Fish Health Service Unit in Halifax helped verify families of fish that were

more resistant to furunculosis. I believe this was great foresight on the part of individuals at the Federation to record these results and follow up on them. These fish may be of great use for hatcheries having to use surface water endemic for furunculosis.

Other topics of interest that the SGRP may want to look at are:

1. What strains of fish are genetically more resistant to Vibrio? What is the cost effectiveness of vaccines and treatments on different genetic strains of fish?

2. The apparent resistance of fry to Infectious Pancreatic Necrosis in New Brunswick Atlantic salmon.

3. On one of our salmon farms in February and March, when water temperatures were between 1.1°C to 3.9°C, a farmer started losing a lot of his fish. Our diagnostic laboratory could not find any bacterial pathogens in these fish. We did notice that all the fish dying were males and were also the biggest fish in the cages. These fish had enlarged testes and some of them had haemorrhages on their testes. Samples sent away for histology revealed reabsorption of spermatic tissue as well as production of spermatic tissue. Since sexual maturation, causing these fish to lose the ability to osmoregulate in waters below 4°C, was probably the cause of death, mortality should be investigated further. This loss of fish was probably both a genetic and environmental problem which should be monitored closely.

4. On a couple of farms last winter there was a high loss of fish due to superchilling. In each pen of approximately 2,500 fish there were one or two survivors. Could these survivors be used for a foundation stock for resistance to cold water? If the SGRP could take fish from this disaster and build them into a cold water resistant stock, it could expand the number of sites for salmon growing in the Bay of Fundy.

In regards to specifics for a disease monitoring program, I would like to outline the program prepared by the New Brunswick Fish Health Advisory Committee to control bacterial kidney disease (Renibacterium salmonarium).

1. Around March of each year, DFO does schedule 3 testing on a sample of all stocks on the aquaculture sites. Testing for Bacterial Kidney Disease and prescreening potential broodstock by fluorescent antibody technique for Renibacterium salmonarium is also conducted at this time.

2. All milt from broodstock is examined prior to spawning by a recognized laboratory using a standard technique.

3. All females are to have their ovarian fluids sampled and tested by a recognized laboratory using a standard technique. All positive egg lots are then destroyed. If indirect fluorescent antibody technique is the accepted testing method, then any BKD organisms identified in 50 fields at 1000X magnification will be counted. Since one BKD organism in 50 fields by IFAT represents ten thousand to ten million BKD organisms per gm of kidney tissue, and possibly the same analogy can be drawn on ovarian fluids, any egg lot testing positive will be destroyed.

4. In the spring of each year, every smolt lot will be tested for BKD. The results of this testing will determine the future of these smolts.

In conclusion, disease resistance is a very worthwhile goal. A disease monitoring program will help define the gains or setbacks of the program based on genetics, disease agents and environment, in order to achieve repeatable strong genetic advances in salmon stocks by selecting for phenotypic advances.

QUESTIONS

John Bailey: To run a selection-for-resistance program, we would have to expose the fish to pathogens. Is this a job for ASF or government or universities?

John O'Halloran: During a furunculosis outbreak, ASF took good advantage of the information. Quarantine facilities are available at DFO, Halifax and the Atlantic Veterinary College in Charlottetown. ASF could set up a system without quarantine and send fish to quarantine units for testing.

Gil Farmer: Are all broodstock sites going to be monitored?

John O'Halloran: Most major producers are testing their fish for pathogens. Education will induce growers to refuse to purchase smolts from unmonitored stock.

Gil Farmer: Who does the testing?

John O'Halloran: New Brunswick has no regulations regarding which laboratories are to be used. This is a weak point and standardization is necessary. This is the topic of upcoming discussions in Halifax.

Floor: How many farms have broodstock?

John O'Halloran: Fourteen, including Arctic charr broodstock.

Richard Saunders: On the topic of freeze-resistant salmon: the danger of superchilling is a problem in the Bay of Fundy area. The dead fish from the superchilling in 1989 were found with mud on their gills, so there is some question as to the cause of death. Plasma freezes between -.8°C and -.9°C. If there are no freeze-resistant proteins, I can't see how much variation there could be in this area. This suggests suffocation to me, possibly due to mud on the gills.

However, the cause in John Malloch's experience was definitely freezing. The fish were stressed by seals, which lowered their tolerance to superchilling.

Roger Doyle: Breeding from the survivors of these episodes has been good. This is a good way to contribute to the domestication of the salmon. However, how can we avoid inbreeding? Is there some way the geneticist can use survivors of such natural disasters, such as disease outbreaks? This is such a simple case of good, natural selection.

John Cornick: There is no program to select remnant survivors of disease. We rely on vaccines to protect artificially selected fish. We don't allow nature to take its course and choose survivors, thereby limiting the severity of the effects of the disease.

Gil Farmer: The first response to a disease outbreak is to treat, thus saving those that would have died. It's just too expensive to let valuable fish die.

Harold Kincaid: A resistant strain of brown trout has been developed, but growers don't want it because stocks are considered still to be carriers and thus not valuable.

John O'Halloran: Furunculosis is a big problem in Scotland. Here, with it being less of a problem, we have been able to control outbreaks at hatcheries by quarantining. Until there's more reliable testing, restriction of transfer between sites is necessary.

Cristina Soto: In some cases, tests can be positive but the infection is at a very low level. Isn't the organism actually present in low levels at most farms anyway? So, since it's not segregated, but everywhere in low levels, we could do something along the lines of general selection for resistance.

John O'Halloran: I don't believe that disease agents are present at every farm. Any pathogen can be eliminated by using specific pathogen-free stock to increase the profitability of salmon farming. Selection for resistance to pathogens is a very good approach for widespread or common diseases.

Robert Cook: During the furunculosis problem in 1984, experts were brought over from Ireland and Scotland. They accepted losses of 20-25 percent as a cost of doing business. We choose to quarantine and avoid high mortalities by carrier testing.

John O'Halloran: This approach has definitely benefitted the industry in New Brunswick, as mortalities are in the range of six to 12 percent.

John Anderson: A big outbreak of furunculosis is under way right now in Norway.

John Cornick: Yes. We're able to contain outbreaks here by monitoring.

Roger Doyle: If there is a general adaptation problem, it is more likely a genetic susceptibility to stress in general. There is no single gene for disease resistance.

John Anderson: There is a practical problem getting a strain resistant to furunculosis, because under no circumstances can we do testing here.

John Cornick: Giles Olivier, DFO Fish Health Service Unit, is overwhelmed by testing requests, since theirs is the only equipment available for testing.

A HEALTH PROGRAM FOR THE SGRP

Dr. Steve Backman, Moore-Clark Canada Inc., St. Andrews, N.B., E0G 2X0.

In the aquaculture industry, the goal of a health monitoring program is to minimize disease and maximize profits. The situation is a little different in the case of the SGRP: because the SGRP is engaged in genetics research, it is necessary to be able to isolate the effects of disease treatments from other effects having a bearing on experimental results. Not only the disease, but the treatment of disease, can affect traits such as weight and growth rates.

It is important to note that diseases are not always caused by an infectious agent. Developmental, nutritional and environmental diseases also occur.

The clinical manifestation of an infectious disease requires the presence of a pathogen and usually involves some alteration in the intricate relationship between the host and environment. Any preventive health program must address all these criteria.

Stress can be loosely defined as any force or environmental change which produces an alteration in the body's homeostasis. The acute stress response is a natural defense mechanism and is a means by which the body attempts to restore homeostasis. This response can be manifested in various ways, including increased perfusion of the gills when oxygen demand is increased or changes in behaviour; for example, to allow escape from a predator.

The intensive rearing conditions which are found in aquaculture operations may induce chronic stress. The stress response may be unsuccessful at resolving pressures such as unremitting disturbances and crowding and may be triggered continually for an extended period of time. Chronic stress response frequently results in physiological changes which may be deleterious, in terms of performance and susceptibility to disease.

Water quality parameters and environmental factors are important considerations when developing a preventive health plan. The influence of environmental factors on disease development is frequently overlooked unless outward clinical signs are evident. Examples of clinical manifestations include gas bubble disease, a result of dissolved gas supersaturation, and methaemoglobinemia, a result of an accumulation of nitrite in the water. Less evident

are the effects of marginal environmental conditions, which, in themselves, may be insufficient to cause clinical signs, but may predispose the fish to developing a disease to which, in other circumstances, it would be resistant. Water quality changes are frequently transient and, therefore, routine monitoring is necessary if any correlation can be made with a disease outbreak.

Diseases caused by infectious agents can negatively affect production in a hatchery. Subclinical infections commonly exist and can reduce overall performance and vitality. Many diseases, such as furunculosis, can have varied clinical manifestations which closely resemble other diseases. It is therefore prudent to ensure all diagnoses be followed up with the appropriate ancillary tests. The hatchery manager must also realize the importance of interpreting reports from regional diagnostic labs in light of the situation on the farm. Costia (Icthyobodo necotor) can get quickly out of control in a hatchery. The diagnosis can often be made quite easily, using simple microscopic techniques. However, when the fish must be shipped to a regional lab, the diagnosis may be missed. That is by no means a reflection on the lab; rather, it is inherent in the nature of the organism. Costia is highly motile and the simple act of placing the fish in a fixative may be sufficient to cause the organism to leave its host. Therefore, it would not be readily apparent when the fish is examined on arrival.

Fungal diseases are quite common in the hatchery. Saprolygnia can occur in first feeding fry. It is usually thought to be related to feeding too large a feed size. The feed gets lodged in the back of the mouth and provides a favourable environment for fungal spores to germinate. The fungi then spread to the surrounding tissue of the mouth, esophagus and gill. It is at this point that it becomes apparent clinically. While treatment is usually successful at reducing mortalities, some of those which survive may develop sufficient scarring to result in constriction of the pneumatic duct. This scarring may manifest itself later in the fish's life as a distended swim bladder. Without knowledge of the previous history of infection, it would be very unlikely that any connection would be made. This example illustrates the importance of accurate record-keeping and the need for a holistic approach to health management.

The awareness that potentially pathogenic organisms may be present in apparently normal fish leads to the question of when to treat. This question is a complex issue and is essentially a cost-benefit analysis of the cost of treating versus the cost of the disease.

Diseases, whether infectious or noninfectious, are best managed through prevention. In a hatchery, this can be accomplished through strict, consistent sanitation procedures and sound husbandry practices. In some situations, this may not be possible or practical, and an alternate approach, aimed at early recognition of potential problems, followed by the application of corrective measures before the problem manifests clinically, may be necessary. In either case, the establishment of an accurate and functional record-keeping system will allow the manager to:

1. Quantify. What are the limitations of the hatchery? What are the water quality parameters? Where are morts occurring and at what age, class or section of the hatchery?

2. Identify. Look for causative agent(s). What management deficiencies exist in the hatchery? Have the water quality parameters changed?

3. Correct. Make changes aimed at reducing mortalities or improving performance. This may include the use of chemotherapeutics or simply modifying present management practices. It is important that any changes implemented are introduced against controls.

4. Evaluate. Perform a cost/benefit analysis on those measures introduced, so that an objective evaluation can be obtained.

QUESTIONS

Gerry Friars: Would you give us an update on vaccinations at low temperature, please?

Steve Backman: Thorburne _et al_. reported good protection in rainbow trout vaccinated I.P. at 2.8°C 5 weeks before transport to brackish water. She also noted that it was 16 weeks before vibriosis occurred in the controls, implying 16 weeks was sufficient for fish vaccinated at low temperature to develop an adequate immune response. It should also be borne in mind that vaccines are no substitute for good husbandry. They are an aid in the prevention of diseases.

Gerry Friars: What about boosters? We make a significant movement of parr at about the 1.8 gm size. Would this be a reasonable time to vaccinate? Water temperature is up at that time.

Steve Backman: It has been reported that fish become immunocompetent at as little as 0.13 gm. However, the duration of immunity from vaccination at this size is very short. In fish weighing more than 1 gm, a higher and more persistent immune response was achieved at 4 gm. A good cut-off would be approximately 3 gm as this would ensure most fish within the population would obtain some benefit from vaccination.

John Cornick: Bill Paterson (Aqua Health) says that 1 gm is the cut-off. It varies from species to species.

Steve Backman: According to Thorburne, there is little difference between results from initial vaccination and the booster when dealing with smaller fish and lower temperatures.

Gil Farmer: Aqua Health recommends an immersion vaccination at the 2 gm size.

John Cornick: A good idea might be to vaccinate at a small size for protection in the hatchery, and then boost to provide protection when moved to the sea cages.

Steve Backman: Paterson says that protection is provided for about 18 months after each vaccination.

John Cornick: Paterson does not promote the use of boosters. He says he has data showing that booster results are insignificant. He feels you are no further ahead with a booster, and that it is not worth the added expense.

Roger Doyle: When talking about genetic studies, it is important to note that diseases are a component of growth. The presence of diseases can mask genetic effects.

Steve Backman: That's why a baseline monitoring program should be created at the site, so that disease information is available when considering effects. Genetic effects can then be considered in light of disease information.

Roger Doyle: Statistical sampling of strains or families can show growth differences that might appear to be physiological, but might be the result of disease.

Cristina Soto: When sampling for health monitoring, is it necessary to kill the fish?

Steve Backman: In many cases, the fish can be sedated and preparations can be obtained from skin and gills for the whole mount and culture. For more detailed diagnostic information, lethal samples must be maintained. Once you have good baseline data, the sample size can be statistically determined and this may be surprisingly small.

Gerry Friars: We are at the mercy of our water supply, which comes from Chamcook Lake. Water intended for SGRP experiments is UV filtered. This is considered to be 95 percent effective. Does dilution reduce the challenge of disease organisms on the fish?

Steve Backman: A qualified yes. In our last discussion, we talked about the relationship between the host, pathogen and environment and anything which provides a positive influence on these factors from the fish's point of view is beneficial. I would also point out that UV sterilizers must be well-maintained, as fouling dramatically cuts their efficiency. Also, if there are 1 billion organisms in a given quantity of water and you kill 95 percent of them, you are still left with 50 million surviving organisms.

Gerry Friars: In the case of BKD, when the trays are lined up downstream from the water supply, will it spread from one to the other via the water supply?

John Cornick: There is little risk when the eggs are water hardened. At that time, the eggs are protected.

Steve Backman: We can't follow up on whether these eggs are still viable. Maybe these end up lost.

Sandy McGeachy: Should we harden all the eggs in the same tank? Do you need a separate tank for each family while hardening?

Rod Carney: Ours are separated until they are hardened, and then they are disinfected.

Bob Cook: There is a good opportunity for a water quality study here, because of the layout of the flow system. The upper area, the first to receive the water, is in production, and the downstream area is research.

Gerry Friars: On the topic of treatments, in the poultry industry it is very standardized. At ASF we mix suppliers of food, drugs, etc., for reasons of cost and convenience. Is this a good or a poor practice.

John O'Halloran: Standardization can provide one more controlled effect, but mixing is a good idea for reasons of avoiding preference problems in feed if it becomes necessary to change the diet.

Sandy McGeachy: Regarding broodstock management and fish health: are you sending primary stock to the demonstration farm?

Gerry Friars: Yes. A back-up stock for each strain is also held at a commercial site.

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